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Human transcription factor IIIC binds to its cognate promoter sequences in a metal coordinated fashion.

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Transcription factor IIIC from human cells (hTFIIIC) contains a 55 kDa polypeptide which specifically binds to the promoter of the VAI and 5S gene This interaction can be abolished by depleting divalent metal cations from the free protein through chelation with EDTA. Prior association of the protein with its DNA-binding sequence renders the complex refractory to chelation by EDTA. Specific binding of hTFIIIC to its cognate promoter sequences--shown by electrophoretic mobility shift and DNase I protection assays--can be restored by the addition of zinc ions. In contrast to the binding of hTFIIIA to the 5S gene, which was monitored in parallel and which exclusively requires Zn2+, the binding of hTFIIIC to the VAI and 5S gene can also be reconstituted--albeit with a lower efficiency--by the transition metals Co2+, Fe2+ and Mn2+ but not by Ni2+ or Cu2+. These results show that hTFIIIC binds to its promoter sequences in a metal coordinated fashion which differs from that observed for the binding of hTFIIIA to the 5S gene.

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Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene.

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Plasmid DNA harbouring the human 5S rRNA gene was assembled into nucleosomes using either Xenopus S150 extracts or purified core histones in the presence of pectin. In both cases reconstitution of nucleosomes led to a complete repression of transcription. This repression could be efficiently counteracted by preincubating the template DNA with highly purified hTFIIIA which allowed the protein to bind to the ICR of the 5S gene. By using an efficient and well-defined in vitro reconstitution system based on isolated core histones in the presence of pectin, which is devoid of endogenous transcription factors, we demonstrate here for the first time that human TFIIIA alone is sufficient to prevent nucleosomal repression of h5S gene transcription and that additional pol III transcription factors are not required to achieve this effect. Additionally, we investigated the binding of hTFIIIA to a mononucleosome reconstituted on the human 5S gene. DNAse footprinting experiments reveal that the entire ICR of the human 5S gene is covered by the nucleosome, thereby precluding the subsequent binding of human TFIIIA to the promoter of the 5S gene.

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